

## EFFECTS OF TEMPERATURE ON THE NUTRITIONAL REQUIREMENTS OF *ARTEMIA SALINA* (L.)

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Details concerning alterations in metabolism at the cellular and subcellular levels induced by temperature are poorly known in invertebrate animals (McWhinnie, 1967); few data exist for crustacea other than on oxygen uptake (Huggins and Munday, 1968). Even though numerous data are available for respiration rates of *Artemia* in relation to temperature (Eliassen, 1952; Grainger, 1956; Conover, 1960; Engel and Angelovic, 1968) conversion of this information into terms of food requirements and energy flow is necessary before making any conclusion (Conover, 1960). At the organismic level, the general effect of temperature is to increase growth rate. This effect has been demonstrated for different *Artemia* strains (Reeve, 1963; Hentschel, 1967; Hentig, 1971). Temperature is one of the major environmental variables which influence metabolic rate. Increasing attention is being paid to the influence of nutritional conditions and to the interactions between temperature and nutrients. Temperature has less influence on the molting frequency of starving *Balanus* when protein or lipids are used instead of carbohydrates (Barnes, Barnes and Finlayson, 1963). In *Carcinus maenas* the metabolic levels observed during starvation are related to the nature of reserves used (Wallace, 1973).

Information is lacking on how temperature affects the nutritional requirements of crustacea. The development of an artificial medium for rearing *Artemia salina* (Utah strain) by Provasoli and d'Agostino (1969) offers a good opportunity to study this problem.

### MATERIALS AND METHODS

Nauplii were hatched at their rearing temperature of  $25^{\circ} \pm 0.5^{\circ} \text{C}$  or  $30^{\circ} \pm 0.5^{\circ} \text{C}$ . After hatching they were kept for 24 hours at  $25^{\circ} \text{C}$  or for 8 hours at  $30^{\circ} \text{C}$  in the hatching medium before being transferred to the nutritive media. In both cases, day one of development is 24 hours after hatching. The growth index achieved on the tenth day of development at  $25^{\circ} \text{C}$  and  $30^{\circ} \text{C}$  was used for graphical comparisons. The action of temperature on starch, adenylic acid (for adenosine monophosphate, AMP) and albumin requirements and on their relative ratios has been studied since salinity has been shown to alter the optimal starch plus AMP/albumin ratio (Hernandorena, 1974b). In the basal medium, this ratio (expressed in mg for 100 ml of media) is  $100 + 60/20$ . The vitamin concentrations were kept constant; they are probably not limiting at  $30^{\circ} \text{C}$  since their concentrations in the basal medium are well above the minimal requirements. However, in view of House's findings (1966a), this may require further investigation. All experiments were done at 24‰ salinity.

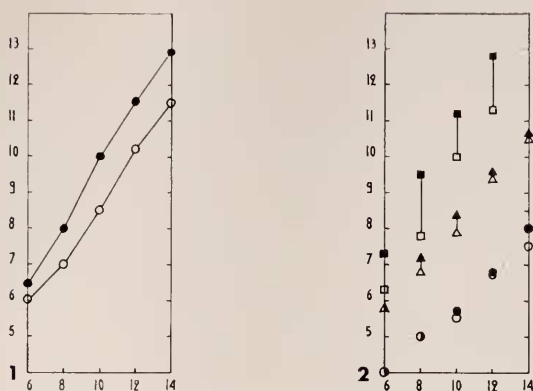


FIGURE 1. Growth rate in relation to temperature using a basal medium. Open circles indicate 25° C; closed circles, 30° C. Abscissa indicates days; ordinate, the growth index.

FIGURE 2. Growth rate in relation to AMP concentration and temperature with starch constant at 100 mg% and albumin constant at 20 mg%. Open symbols indicate 25° C; closed symbols, 30° C. Circles show AMP at 20 mg%; triangles, AMP at 40 mg%; squares, AMP at 100 mg%. Abscissa indicates days; ordinate, the growth index.

### RESULTS

When using the same basal medium, the growth rate at 30° C was higher than the growth rate at 25° C (Fig. 1). These results indicate that the effect of tem-

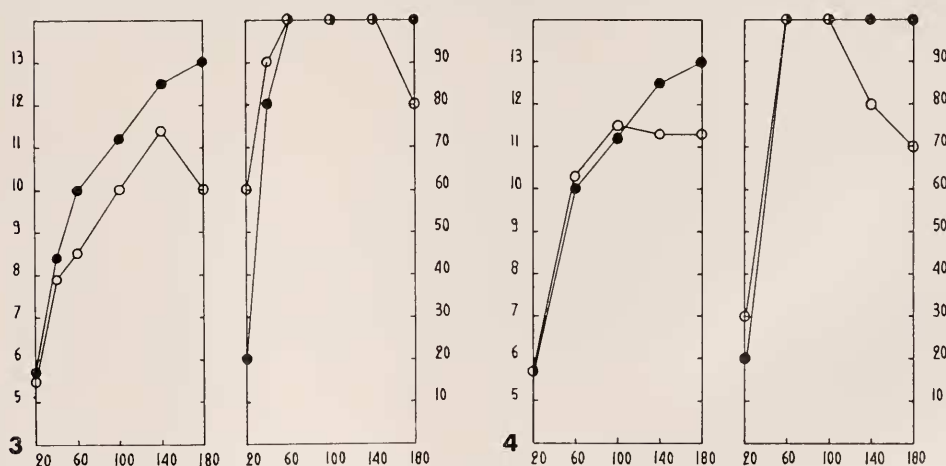


FIGURE 3. Effect of AMP requirement in relation to temperature; both abscissae represent AMP in mg%; starch constant at 100 mg%; albumin constant at 20 mg%. Open circles represent experiments at 25° C; closed circles, those at 30° C. In this and subsequent figures, growth is represented in the left-hand and survival in the right-hand graph, the left-hand ordinate being the growth index for the tenth day of development, and the right-hand ordinate being the survival percentage for index 10 (at the end of larval life).

FIGURE 4. Effect of AMP/starch ratio at 30° C; both abscissae represent AMP in mg%; albumin constant at 20 mg%. Open circles represent starch at 200 mg%; closed circles, starch at 100 mg%. The left and right ordinates are for growth and survival as in Figure 3.

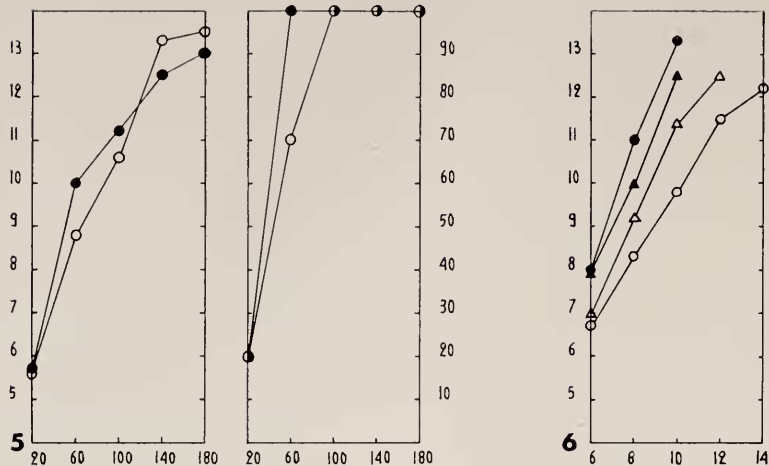


FIGURE 5. Effect of AMP/albumin ratio at 30° C; both abscissae represent AMP in mg%; starch constant at 100 mg%. Open circles represent albumin at 60 mg%; closed circles, albumin at 20 mg%. The left and right ordinates are for growth and survival as in Figure 3.

FIGURE 6. Growth rate in relation to AMP/albumin ratio and temperature, with starch constant at 100 mg% and AMP constant at 140 mg%. Open symbols indicate 25° C; closed symbols, 30° C. Circles represent albumin at 60 mg%; triangles, albumin at 20 mg%. Abscissa indicates days; ordinate, the growth index.

perature depends on the available quantities and ratios of AMP, albumin and starch.

### AMP requirement

With starch constant at 100 mg% and albumin constant at 20 mg, the difference between growth rate at 25° C and 30° C increased with increasing AMP concentration up to 100 mg% (Fig. 2). AMP deficiency is more detrimental at 30° C than at 25° C. Growth rate increased with increasing AMP concentration up to a 140 mg% level at 25° C and to a 180 mg% level at 30° C (Fig. 3). With increasing AMP concentration above 100 mg%, additional starch became detrimental (Fig. 4) and additional albumin beneficial (Fig. 5). The difference between growth rate at 25° C and 30° C increased with increasing AMP concentration above 100 mg% provided additional albumin was supplied (Fig. 6).

### Albumin requirement

With AMP constant at 60 mg% and starch constant at 100 mg% optimal albumin concentration is 20 mg% at 25° C and 30° C, but albumin excess is less detrimental at 30° C than at 25° C (Fig. 7). At 30° C additional starch was detrimental in an albumin deficient medium and beneficial with increasing albumin concentration; however, best growth is achieved with a 100 mg% starch concentration and a 180 mg% AMP concentration, whatever the albumin concentration (Fig. 8).

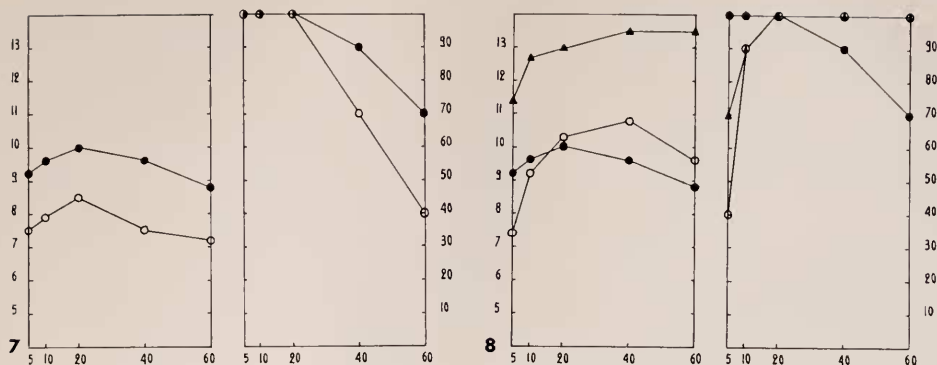


FIGURE 7. Effect of albumin requirement in relation to temperature; both abscissae represent albumin in mg%; starch constant at 100 mg%; AMP constant at 60 mg%. Open circles indicate 25° C; closed circles, 30° C; and the left and right ordinates are for growth and survival as in Figure 3.

FIGURE 8. Effects of AMP/albumin ratio and starch/albumin ratio at 30° C; both abscissae represent albumin in mg%. Open circles represent AMP at 60 mg%, starch at 200 mg%; closed circles, AMP at 60 mg%, starch at 100 mg%; and closed triangles, AMP at 180 mg%, starch at 100 mg%. Ordinates are for growth and survival as in Figure 3.

### Starch requirement

With AMP constant at 60 mg% and albumin constant at 20 mg%, optimal starch concentration is 100 mg% at 25° C and less well-defined at 30° C (Fig. 9). However starch deficiency was less detrimental at 30° C than at 25° C. Additional

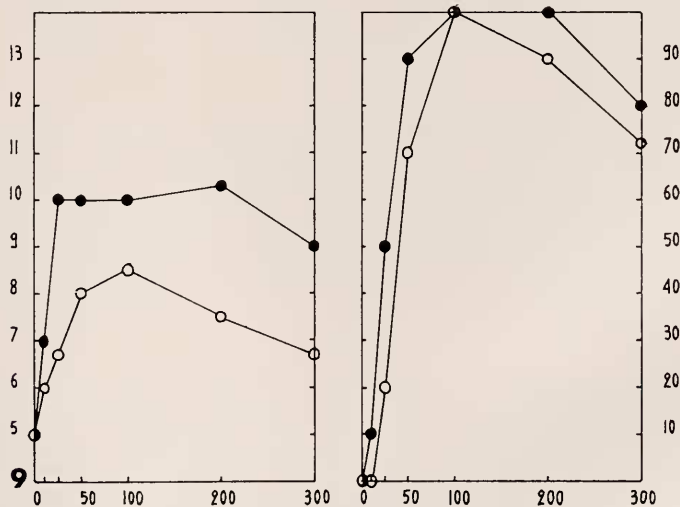


FIGURE 9. Effect of starch requirement in relation to temperatures; both abscissae represent starch in mg%; albumin constant at 20 mg%; AMP constant at 60 mg%. Open circles indicate 25° C; closed circles, 30° C. The left and right ordinates are for growth and survival as in Figure 3.

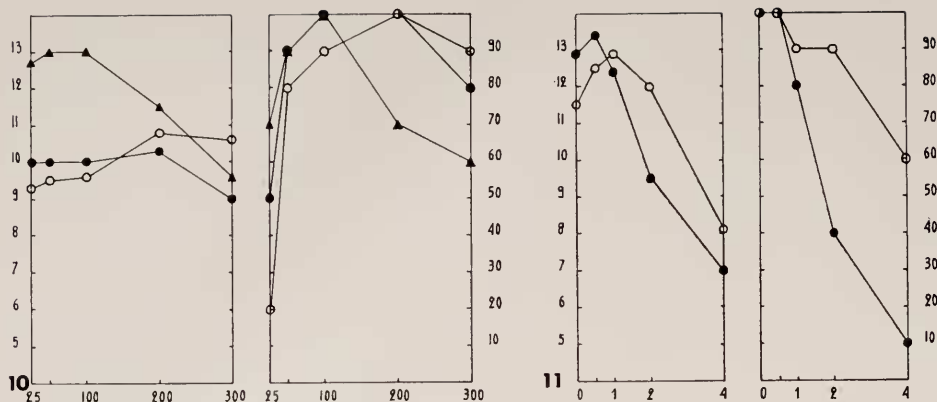


FIGURE 10. Effects of AMP/starch ratio and starch/albumin ratio at 30° C; both abscissae represent starch in mg%. Open circles represent AMP at 60 mg%, albumin at 40 mg%; closed circles, AMP at 60 mg%, albumin at 20 mg%; and closed triangles, AMP at 180 mg%, albumin at 20 mg%. The left and right ordinates are for growth and survival as in Figure 3.

FIGURE 11. Effect of lecithin requirement in relation to temperature; both abscissae represent lecithin in mg%; basal medium used. Open circles represent experiments at 25° C; closed circles, those at 30° C. The left ordinates represent growth, in this case as the index for the fourteenth day of development; the right ordinates being survival as in Figure 3.

albumin was detrimental in a starch deficient medium and beneficial with increasing starch concentration, but additional AMP was detrimental with increasing starch concentration (Fig. 10). Best growth is achieved with a 20 mg% albumin concentration and a 180 mg% AMP concentration provided additional starch was not supplied.

### Lecithin requirement

With starch constant at 100 mg%, AMP constant at 60 mg% and albumin at 20 mg%, the effect of additional lecithin depends on temperature. This effect is more obvious on the fourteenth day of development (Fig. 11). Growth rate increased with increasing lecithin concentration up to a 1 mg% concentration at 25° C and up to a 0.5 mg% concentration at 30° C. Excess of lecithin is more detrimental at 30° C than at 25° C. Additional data on the effect of lecithin at 30° C will be presented elsewhere (Hernandorena, in preparation).

### DISCUSSION

In *Artemia*, increasing the temperature from 25° C to 30° C resulted in a different optimal starch plus AMP/albumin ratio. At 30° C maximal growth is achieved with more AMP and less starch or more albumin than at 25° C. At 25° C and with increasing salinity, less AMP, less starch, and more albumin are required for optimal growth (Hernandorena, in preparation). The temperature-salinity relationship defined by Kinne (1963, for review) is evident during post-embryonic development in *Artemia*; that is, when salinity increases optimal temperature decreases (Hentig, 1971). Similarly, the temperature effect on the

quantitative requirement for AMP is opposite to the effect of salinity, indicating that in *Artemia* the AMP requirement could be the biochemical basis for the temperature-salinity relationship.

The first conclusion to be drawn is that nutritional requirements have to be defined in relation to temperature and salinity. This emphasizes the necessity for marine ecologists to start nutritional studies and to integrate the nutritional parameter.

Now the problem is to relate the temperature-induced effects on nutritional requirements to metabolism. According to Sang (1959) the balance between nutrients deserves more investigation because here one delves most closely into the examination of the metabolic processes. The information available concerning the action of temperature centers around utilization of energy producing nutrients and energy metabolism.

In insects few data are available on the influence of specific nutrients in relation to temperature. In *Periplaneta americana* the conversion efficiency (energy stored/energy input) is temperature, sex and food dependent (Prema, 1971). In *Pseudosarcophaga affinis* a nutrient balance rich in glucose was relatively beneficial in cold and detrimental in warmth (House, 1966b). The relative food value of the two synthetic diets was inverted between 15° C and 30° C. The nutrients responsible of this inversion were not defined, but the RNA content of the diets had been kept constant (House, 1972).

In *Drosophila*, temperature-dependent changes in respiration rate resulted from changes in the substrates feeding into the Krebs cycle (Burr and Hunter, 1970). Anders, Drawert, Anders and Reuther (1964) have demonstrated that the level of free amino acids is inversely related to environmental temperature in *Drosophila*. In the crayfish, low temperature acclimation resulted in measurable changes in the ratio of biochemical pathways involved in glucose utilization. Amino acid synthesis would remove intermediates from the carbohydrate cycle (McWhinnie and O'Connor, 1967).

The relative contribution of carbohydrates, lipids and proteins to energy production during the embryonic development of *Artemia* has been studied under different experimental conditions. The relative contribution of carbohydrates to energy production decreases with increasing energy drain. Lipids constitute the main energy reserve, and their utilization increases with increasing energy requirements. The contribution of proteins depends essentially on salinity (Hentig, 1971). These data concern the embryonic development which is a closed system independent of nutrition.

In *Artemia* the action of temperature must involve AMP metabolism. Temperature elevation does not increase growth rate in an AMP deficient medium. So the temperature-induced increase in growth rate is mediated through AMP metabolism. At 25° C and 24 ‰ salinity, AMP and energetic nutrient requirements increase with increasing albumin concentration in the diet (Hernandorena, 1974b). This result can be interpreted as an increased ATP requirement for protein biosynthesis since ATP produced by the energetic metabolism is necessary for amino acid incorporation into tissue proteins. Puromycin inhibition depends, in fact, on the AMP and the albumin concentrations of the diet (Hernandorena, 1975). At 30° C and 24‰ salinity, growth rate increases with increasing AMP

and albumin concentration without an additional energetic nutrient requirement. Moreover, increasing starch or lecithin concentration is detrimental. It can be assumed that the relevant discrepancy between nutritional requirements at 25° C and 30° C is due to the AMP/energetic nutrient ratio.

Besides gathering metabolic data, a further object of these studies is to understand the morphogenetic significance of the dietary AMP requirement. AMP deficiency induces a supernumerary gonopode morphogenesis (Hernandorena, 1970, 1972, 1974a). It is interesting to note in view of the findings that the AMP requirement increases with temperature, and that thermal stress induced a supernumerary genital appendage in mosquitoes (Horsfall and Anderson, 1963) and one or two pairs of supernumerary appendages in *Glomeris marginata* (Juperthie-Jupeau, 1971).

Metabolic adaptations to nutritional conditions, temperature and salinity were shown to affect the quantitative AMP requirement. Hence AMP-induced morphogenesis would result from a slight shift in the balance of metabolic pathways rather than from the blocking of an essential reaction (Hernandorena, 1975).

#### SUMMARY

1. The growth rate of *Artemia* in a uniform basic medium is faster at 30° C than at 25° C.
2. Temperature and salinity have opposite effects on the quantitative requirement for AMP.
3. At 30° C maximal growth is achieved with more AMP, more albumin, and less starch than at 25° C.
4. The effects of temperature are mediated by the ratio between energetic nutrients and AMP.

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